



Nonprovisional Patent Application

17355CIP3 (BOT)

Steward, L. E. et al., Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins

SEQUENCE LISTING

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<120> Clostridial Neurotoxin Compositions and
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<140> US 10/757,077
<141> 2004-01-14

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<150> US 10/163,106
<151> 2003-06-04

<160> 83

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Ala Asp Thr Gln Val Leu Met
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<223> Xaa is any amino acid.

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<223> Xaa is any amino acid.

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<223> Xaa is any hydrophobic amino acid.

<400> 24

Tyr Xaa Xaa Xaa

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<210> 25

<211> 4

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<213> Clostridium botulinum sertotype A

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Lys Ala Phe Lys

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<210> 26

<211> 6
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<213> Clostridium botulinum sertotype A

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1 5

<210> 27
<211> 8
<212> PRT
<213> Clostridium botulinum serotype A

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Pro Phe Val Asn Lys Gln Phe Asn
1 5

<210> 28
<211> 22
<212> PRT
<213> Clostridium botulinum sertotype A

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Gly Ile Ile Thr Ser Lys
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<210> 29
<211> 438
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<213> Clostridium botulinum sertotype A

<400> 29
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1 5 10 15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
85 90 95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
130 135 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
 145 150 155 160
 Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
 165 170 175
 Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
 180 185 190
 Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
 195 200 205
 Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
 210 215 220
 Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
 225 230 235 240
 Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
 245 250 255
 Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
 260 265 270
 Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
 275 280 285
 Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
 290 295 300
 Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
 305 310 315 320
 Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
 325 330 335
 Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
 340 345 350
 Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
 355 360 365
 Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
 370 375 380
 Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
 385 390 395 400
 Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
 405 410 415
 Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
 420 425 430
 Gly Ile Ile Thr Ser Lys
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<210> 30

<211> 441

<212> PRT

<213> Clostridium botulinum sertotype B

<400> 30

Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
 1 5 10 15
 Asn Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg
 20 25 30
 Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
 35 40 45
 Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
 50 55 60
 Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn

65	70	75	80
Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe			
	85	90	95
Asn Arg Ile Lys Ser Lys Pro Leu Gly Glu Lys Leu Leu Glu Met Ile			
	100	105	110
Ile Asn Gly Ile Pro Tyr Leu Gly Asp Arg Arg Val Pro Leu Glu Glu			
	115	120	125
Phe Asn Thr Asn Ile Ala Ser Val Thr Val Asn Lys Leu Ile Ser Asn			
	130	135	140
Pro Gly Glu Val Glu Arg Lys Lys Gly Ile Phe Ala Asn Leu Ile Ile			
	145	150	155
Phe Gly Pro Gly Pro Val Leu Asn Glu Asn Glu Thr Ile Asp Ile Gly			
	165	170	175
Ile Gln Asn His Phe Ala Ser Arg Glu Gly Phe Gly Gly Ile Met Gln			
	180	185	190
Met Lys Phe Cys Pro Glu Tyr Val Ser Val Phe Asn Asn Val Gln Glu			
	195	200	205
Asn Lys Gly Ala Ser Ile Phe Asn Arg Arg Gly Tyr Phe Ser Asp Pro			
	210	215	220
Ala Leu Ile Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr			
	225	230	235
Gly Ile Lys Val Asp Asp Leu Pro Ile Val Pro Asn Glu Lys Lys Phe			
	245	250	255
Phe Met Gln Ser Thr Asp Ala Ile Gln Ala Glu Glu Leu Tyr Thr Phe			
	260	265	270
Gly Gly Gln Asp Pro Ser Ile Ile Thr Pro Ser Thr Asp Lys Ser Ile			
	275	280	285
Tyr Asp Lys Val Leu Gln Asn Phe Arg Gly Ile Val Asp Arg Leu Asn			
	290	295	300
Lys Val Leu Val Cys Ile Ser Asp Pro Asn Ile Asn Ile Asn Ile Tyr			
	305	310	315
Lys Asn Lys Phe Lys Asp Lys Tyr Lys Phe Val Glu Asp Ser Glu Gly			
	325	330	335
Lys Tyr Ser Ile Asp Val Glu Ser Phe Asp Lys Leu Tyr Lys Ser Leu			
	340	345	350
Met Phe Gly Phe Thr Glu Thr Asn Ile Ala Glu Asn Tyr Lys Ile Lys			
	355	360	365
Thr Arg Ala Ser Tyr Phe Ser Asp Ser Leu Pro Pro Val Lys Ile Lys			
	370	375	380
Asn Leu Leu Asp Asn Glu Ile Tyr Thr Ile Glu Glu Gly Phe Asn Ile			
	385	390	395
Ser Asp Lys Asp Met Glu Lys Glu Tyr Arg Gly Gln Asn Lys Ala Ile			
	405	410	415
Asn Lys Gln Ala Tyr Glu Glu Ile Ser Lys Glu His Leu Ala Val Tyr			
	420	425	430
Lys Ile Gln Met Cys Lys Ser Val Lys			
	435	440	

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Tyr Ile Lys Ile
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Tyr Asp Ser Thr
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Tyr Gly Ser Thr
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Tyr Asn Lys Phe
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Tyr Leu Asn Phe
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<400> 37
Tyr Asp Gly Phe
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<400> 38
Tyr Lys Leu Leu
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<223> Amino terminal 30 amino acids of light chain

<400> 39
Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met
20 25 30

<210> 40
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<213> Clostridium botulinum serotype A

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<222> (1)...(50)
<223> Carboxyl terminal 50 amino acids of light chain

<400> 40
Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn Gly Gln
1 5 10 15
Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu Lys Asn Phe Thr
20 25 30
Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg Gly Ile Ile Thr
35 40 45
Ser Lys
50

<210> 41
<211> 30
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<213> Clostridium botulinum serotype B

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<222> (13)...(30)
<223> Amino terminal 30 amino acids of light chain

<400> 41
Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
1 5 10 15
Asp Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr
20 25 30

<210> 42
<211> 50
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<213> Clostridium botulinum serotype B

<220>
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<223> Carboxyl terminal 50 amino acids of light chain

<400> 42
Tyr Thr Ile Glu Glu Gly Phe Asn Ile Ser Asp Lys Asn Met Gly Lys
1 5 10 15
Glu Tyr Arg Gly Gln Asn Lys Ala Ile Asn Lys Gln Ala Tyr Glu Glu
20 25 30
Ile Ser Lys Glu His Leu Ala Val Tyr Lys Ile Gln Met Cys Lys Ser
35 40 45
Val Lys
50

<210> 43
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Lys Asn Ile Leu Tyr Leu Asp Thr His Leu Asn Thr Leu Ala
20 25 30

<210> 44
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1 5 10 15
Ser Arg Asn Pro Ala Leu Arg Lys Val Asn Pro Glu Asn Met Leu Tyr
20 25 30
Leu Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr
35 40 45
Asn Lys
50

<210> 45
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<212> PRT
<213> Clostridium botulinum serotype D

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<223> Amino terminal 30 amino acids of light chain

<400> 45
Met Thr Trp Pro Val Lys Asp Phe Asn Tyr Ser Asp Pro Val Asn Asp
1 5 10 15
Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile
20 25 30

<210> 46
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Tyr Thr Ile Arg Asp Gly Phe Asn Leu Thr Asn Lys Gly Phe Asn Ile
1 5 10 15
Glu Asn Ser Gly Gln Asn Ile Glu Arg Asn Pro Ala Leu Gln Lys Leu
20 25 30

Ser Ser Glu Ser Val Val Asp Leu Phe Thr Lys Val Cys Leu Arg Leu
35 40 45
Thr Lys
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Met Pro Lys Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp Arg
1 5 10 15
Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Tyr
20 25 30

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Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe Arg Gly Gln Asn Ala
1 5 10 15
Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr Gly Arg Gly Leu Val
20 25 30
Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val Ser Val Lys Gly Ile
35 40 45
Arg Lys
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Met Pro Val Ala Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp
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Asp Thr Ile Leu Tyr Met Gln Ile Pro Tyr Glu Glu Lys Ser
20 25 30

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Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn Asn Arg
1 5 10 15
Gly Gln Ser Ile Lys Leu Asn Pro Lys Ile Ile Asp Ser Ile Pro Asp
20 25 30
Lys Gly Leu Val Glu Lys Ile Val Lys Phe Cys Lys Ser Val Ile Pro
35 40 45
Arg Lys
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<211> 30
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Met Pro Val Asn Ile Lys Asn Phe Asn Tyr Asn Asp Pro Ile Asn Asn
1 5 10 15
Asp Asp Ile Ile Met Met Glu Pro Phe Asn Asp Pro Gly Pro
20 25 30

<210> 52
<211> 50
<212> PRT
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<223> Carboxyl terminal 50 amino acids of light chain

<400> 52

Gln Asn Glu Gly Phe Asn Ile Ala Ser Lys Asn Leu Lys Thr Glu Phe
1 5 10 15
Asn Gly Gln Asn Lys Ala Val Asn Lys Glu Ala Tyr Glu Glu Ile Ser
20 25 30
Leu Glu His Leu Val Ile Tyr Arg Ile Ala Met Cys Lys Pro Val Met
35 40 45
Tyr Lys
50

<210> 53

<211> 30

<212> PRT

<213> Clostridium botulinum serotype A

<220>

<221> DOMAIN

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<223> Amino terminal 30 amino acids of light chain

<221> VARIANT

<222> (4)...(4)

<223> Alanine substitution

<400> 53

Met Pro Phe Ala Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
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Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met
20 25 30

<210> 54

<211> 50

<212> PRT

<213> Clostridium botulinum serotype A

<220>

<221> DOMAIN

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<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (25)...(25)

<223> Arginine substitution

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Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn Gly Gln
1 5 10 15
Asn Thr Glu Ile Asn Asn Met Asn Arg Thr Lys Leu Lys Asn Phe Thr
20 25 30
Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg Gly Ile Ile Thr
35 40 45
Ser Lys
50

<210> 55
<211> 30
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<220>
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<222> (1)...(30)
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT
<222> (10)...(10)
<223> Lysine substitution

<400> 55
Met Pro Phe Val Asn Lys Gln Phe Asn Lys Lys Asp Pro Val Asn Gly
1 5 10 15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met
20 25 30

<210> 56
<211> 50
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<221> VARIANT
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<221> VARIANT
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<223> Alanine substitution

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Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn Gly Gln
1 5 10 15
Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu Lys Asn Ala Ala
20 25 30
Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg Gly Ile Ile Thr
35 40 45
Ser Lys
50

<210> 57
<211> 30
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<213> Clostridium botulinum serotype A

<220>
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<223> Amino terminal 30 amino acids of light chain

<221> VARIANT
<222> (21)...(21)
<223> Arginine substitution

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1 5 10 15
Val Asp Ile Ala Arg Ile Lys Ile Pro Asn Ala Gly Gln Met
20 25 30

<210> 58
<211> 50
<212> PRT
<213> Clostridium botulinum serotype A

<220>
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<222> (1)...(50)
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT
<222> (13)...(13)
<223> Histidine substitution

<400> 58
Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn His Asn Gly Gln
1 5 10 15
Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu Lys Asn Phe Thr
20 25 30
Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg Gly Ile Ile Thr
35 40 45
Ser Lys
50

<210> 59
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<222> (1)...(30)
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT
<222> (7)...(7)
<223> Histidine substitution

<400> 59

Met Pro Phe Val Asn Lys His Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met
20 25 30

<210> 60

<211> 50

<212> PRT

<213> Clostridium botulinum serotype A

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (43)...(43)

<223> Alanine substitution

<400> 60

Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn Gly Gln
1 5 10 15
Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu Lys Asn Phe Thr
20 25 30
Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Ala Arg Gly Ile Ile Thr
35 40 45
Ser Lys
50

<210> 61

<211> 30

<212> PRT

<213> Clostridium botulinum serotype B

<220>

<221> DOMAIN

<222> (1)...(30)

<223> Amino terminal 30 amino acids of light chain

<221> CONFLICT

<222> (3)...(3)

<223> Alanine substitution

<400> 61

Met Pro Ala Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
1 5 10 15
Asp Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr
20 25 30

<210> 62

<211> 50

<212> PRT

<213> Clostridium botulinum serotype B

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (44)...(44)

<223> Arginine substitution

<400> 62

Tyr Thr Ile Glu Glu Gly Phe Asn Ile Ser Asp Lys Asn Met Gly Lys

1

5

10

15

Glu Tyr Arg Gly Gln Asn Lys Ala Ile Asn Lys Gln Ala Tyr Glu Glu

20

25

30

Ile Ser Lys Glu His Leu Ala Val Tyr Lys Ile Arg Met Cys Lys Ser

35

40

45

Val Lys

50

<210> 63

<211> 30

<212> PRT

<213> Clostridium botulinum serotype B

<220>

<221> DOMAIN

<222> (1)...(30)

<223> Amino terminal 30 amino acids of light chain

<221> VARIANT

<222> (21)...(21)

<223> Alanine substitution

<221> VARIANT

<222> (22)...(22)

<223> Alanine substitution

<400> 63

Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn

1

5

10

15

Asp Asn Ile Ile Ala Ala Glu Pro Pro Phe Ala Arg Gly Thr

20

25

30

<210> 64

<211> 50

<212> PRT

<213> Clostridium botulinum serotype B

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (41)...(41)

<223> Arginine substitution

<400> 64

Tyr Thr Ile Glu Glu Gly Phe Asn Ile Ser Asp Lys Asn Met Gly Lys
1 5 10 15
Glu Tyr Arg Gly Gln Asn Lys Ala Ile Asn Lys Gln Ala Tyr Glu Glu
20 25 30
Ile Ser Lys Glu His Leu Ala Val Arg Lys Ile Gln Met Cys Lys Ser
35 40 45
Val Lys
50

<210> 65

<211> 30

<212> PRT

<213> Clostridium botulinum serotype B

<220>

<221> DOMAIN

<222> (1)...(30)

<223> Amino terminal 30 amino acids of light chain

<221> VARIANT

<222> (10)...(10)

<223> Arginine substitution

<400> 65

Met Pro Val Thr Ile Asn Asn Phe Asn Arg Asn Asp Pro Ile Asp Asn
1 5 10 15
Asp Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr
20 25 30

<210> 66

<211> 50

<212> PRT

<213> Clostridium botulinum serotype B

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (30)...(30)

<223> Lysine substitution

<400> 66

Tyr Thr Ile Glu Glu Gly Phe Asn Ile Ser Asp Lys Asn Met Gly Lys
1 5 10 15
Glu Tyr Arg Gly Gln Asn Lys Ala Ile Asn Lys Gln Ala Lys Glu Glu
20 25 30
Ile Ser Lys Glu His Leu Ala Val Tyr Lys Ile Gln Met Cys Lys Ser
35 40 45
Val Lys
50

<210> 67

<211> 30

<212> PRT

<213> Clostridium botulinum serotype C1

<220>

<221> DOMAIN

<222> (1)...(30)

<223> Amino terminal 30 amino acids of light chain

<221> VARIANT

<222> (8)...(8)

<223> Lysine substitution

<400> 67

Met Pro Ile Thr Ile Asn Asn Lys Asn Tyr Ser Asp Pro Val Asp Asn
1 5 10 15
Lys Asn Ile Leu Tyr Leu Asp Thr His Leu Asn Thr Leu Ala
20 25 30

<210> 68

<211> 50

<212> PRT

<213> Clostridium botulinum serotype C1

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (48)...(48)

<223> Arginine substitution

<400> 68

Asn Ile Pro Lys Ser Asn Leu Asn Val Leu Phe Met Gly Gln Asn Leu
1 5 10 15
Ser Arg Asn Pro Ala Leu Arg Lys Val Asn Pro Glu Asn Met Leu Tyr
20 25 30
Leu Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Arg
35 40 45
Asn Lys
50

<210> 69
<211> 30
<212> PRT
<213> Clostridium botulinum serotype D

<220>
<221> DOMAIN
<222> (1)...(30)
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT
<222> (5)...(5)
<223> Alanine substitution

<221> VARIANT
<222> (14)...(14)
<223> Alanine substitution

<400> 69
Met Thr Trp Pro Ala Lys Asp Phe Asn Tyr Ser Asp Pro Ala Asn Asp
1 5 10 15
Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile
20 25 30

<210> 70
<211> 50
<212> PRT
<213> Clostridium botulinum serotype D

<220>
<221> DOMAIN
<222> (1)...(50)
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT
<222> (44)...(44)
<223> Alanine substitution

<400> 70
Tyr Thr Ile Arg Asp Gly Phe Asn Leu Thr Asn Lys Gly Phe Asn Ile
1 5 10 15
Glu Asn Ser Gly Gln Asn Ile Glu Arg Asn Pro Ala Leu Gln Lys Leu
20 25 30
Ser Ser Glu Ser Val Val Asp Leu Phe Thr Lys Ala Cys Leu Arg Leu
35 40 45
Thr Lys
50

<210> 71
<211> 30
<212> PRT
<213> Clostridium botulinum serotype E

<220>

<221> DOMAIN

<222> (1)...(30)

<223> Amino terminal 30 amino acids of light chain

<221> VARIANT

<222> (13)...(13)

<223> Alanine substitution

<400> 71

Met	Pro	Lys	Ile	Asn	Ser	Phe	Asn	Tyr	Asn	Asp	Pro	Ala	Asn	Asp	Arg
1						5			10					15	
Thr	Ile	Leu	Tyr	Ile	Lys	Pro	Gly	Gly	Cys	Gln	Glu	Phe	Tyr		
					20				25				30		

<210> 72

<211> 50

<212> PRT

<213> Clostridium botulinum serotype E

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (31)...(31)

<223> Histidine substitution

<400> 72

Gly	Tyr	Asn	Ile	Asn	Asn	Leu	Lys	Val	Asn	Phe	Arg	Gly	Gln	Asn	Ala
1						5			10				15		
Asn	Leu	Asn	Pro	Arg	Ile	Ile	Thr	Pro	Ile	Thr	Gly	Arg	Gly	His	Val
					20				25				30		
Lys	Lys	Ile	Ile	Arg	Phe	Cys	Lys	Asn	Ile	Val	Ser	Val	Lys	Gly	Ile
					35			40				45			
Arg	Lys														
	50														

<210> 73

<211> 30

<212> PRT

<213> Clostridium botulinum serotype E

<220>

<221> DOMAIN

<222> (1)...(30)

<223> Amino terminal 30 amino acids of light chain

<221> VARIANT

<222> (7)...(7)

<223> Arginine substitution

<400> 73
Met Pro Lys Ile Asn Ser Arg Asn Tyr Asn Asp Pro Val Asn Asp Arg
1 5 10 15
Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Tyr
20 25 30

<210> 74
<211> 50
<212> PRT
<213> Clostridium botulinum serotype E

<220>
<221> DOMAIN
<222> (1)...(50)
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT
<222> (42)...(42)
<223> Alanine substitution

<221> VARIANT
<222> (43)...(43)
<223> Alanine substitution

<400> 74
Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe Arg Gly Gln Asn Ala
1 5 10 15
Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr Gly Arg Gly Leu Val
20 25 30
Lys Lys Ile Ile Arg Phe Cys Lys Asn Ala Ala Ser Val Lys Gly Ile
35 40 45
Arg Lys
50

<210> 75
<211> 30
<212> PRT
<213> Clostridium botulinum serotype E

<220>
<221> DOMAIN
<222> (1)...(30)
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT
<222> (30)...(30)
<223> Arginine substitution

<400> 75
Met Pro Lys Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp Arg
1 5 10 15
Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Arg

20

25

30

<210> 76

<211> 50

<212> PRT

<213> Clostridium botulinum serotype E

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (45)...(45)

<223> Alanine substitution

<400> 76

Gly	Tyr	Asn	Ile	Asn	Asn	Leu	Lys	Val	Asn	Phe	Arg	Gly	Gln	Asn	Ala
1				5				10					15		
Asn	Leu	Asn	Pro	Arg	Ile	Ile	Thr	Pro	Ile	Thr	Gly	Arg	Gly	Leu	Val
			20				25					30			
Lys	Lys	Ile	Ile	Arg	Phe	Cys	Lys	Asn	Ile	Val	Ser	Ala	Lys	Gly	Ile
		35				40					45				
Arg	Lys														
	50														

<210> 77

<211> 30

<212> PRT

<213> Clostridium botulinum serotype F

<220>

<221> DOMAIN

<222> (1)...(30)

<223> Amino terminal 30 amino acids of light chain

<221> VARIANT

<222> (3)...(3)

<223> Alanine substitution

<400> 77

Met	Pro	Ala	Ala	Ile	Asn	Ser	Phe	Asn	Tyr	Asn	Asp	Pro	Val	Asn	Asp
1					5				10				15		
Asp	Thr	Ile	Leu	Tyr	Met	Gln	Ile	Pro	Tyr	Glu	Glu	Lys	Ser		
					20			25				30			

<210> 78

<211> 50

<212> PRT

<213> Clostridium botulinum serotype F

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (46)...(46)

<223> Alanine substitution

<400> 78

Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn Asn Arg
1 5 10 15
Gly Gln Ser Ile Lys Leu Asn Pro Lys Ile Ile Asp Ser Ile Pro Asp
20 25 30
Lys Gly Leu Val Glu Lys Ile Val Lys Phe Cys Lys Ser Ala Ile Pro
35 40 45
Arg Lys
50

<210> 79

<211> 30

<212> PRT

<213> Clostridium botulinum serotype G

<220>

<221> DOMAIN

<222> (1)...(30)

<223> Amino terminal 30 amino acids of light chain

<221> VARIANT

<222> (8)...(8)

<223> Histidine substitution

<400> 79

Met Pro Val Asn Ile Lys Asn His Asn Tyr Asn Asp Pro Ile Asn Asn
1 5 10 15
Asp Asp Ile Ile Met Met Glu Pro Phe Asn Asp Pro Gly Pro
20 25 30

<210> 80

<211> 50

<212> PRT

<213> Clostridium botulinum serotype G

<220>

<221> DOMAIN

<222> (1)...(50)

<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT

<222> (47)...(47)

<223> Alanine substitution

<400> 80

Gln Asn Glu Gly Phe Asn Ile Ala Ser Lys Asn Leu Lys Thr Glu Phe
1 5 10 15
Asn Gly Gln Asn Lys Ala Val Asn Lys Glu Ala Tyr Glu Glu Ile Ser
20 25 30
Leu Glu His Leu Val Ile Tyr Arg Ile Ala Met Cys Lys Pro Ala Met
35 40 45
Tyr Lys
50

<210> 81
<211> 50
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(50)
<223> Peptide comprising a 6x His tag and S-tag

<400> 81
Met His His His His His Ser Ser Gly Leu Val Pro Arg Gly Ser
1 5 10 15
Gly Met Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp
20 25 30
Ser Pro Asp Leu Gly Thr Asp Asp Asp Lys Ala Met Tyr Lys Asp
35 40 45
Pro Val
50

<210> 82
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(14)
<223> Peptide comprising a 6x His tag

<400> 82
Asn Phe Thr Lys Leu Thr Arg Ala His His His His His
1 5 10

<210> 83
<211> 59
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(59)
<223> Peptide comprising a 6x His tag and S-tag

<400> 83

Met	His	His	His	His	His	Ser	Ser	Gly	Leu	Val	Pro	Arg	Gly	Ser		
1						5			10			15				
Gly	Met	Lys	Glu	Glu	Thr	Ala	Ala	Ala	Lys	Phe	Glu	Arg	Gln	His	Met	Asp
						20			25			30				
Ser	Pro	Asp	Leu	Gly	Thr	Asp	Asp	Asp	Lys	Ala	Met	Gly	Ser	Phe		
						35			40			45				
Val	Asn	Lys	Gln	Phe	Asn	Tyr	Lys	Asp	Asp	Pro	Val					
						50			55							